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A SEARCH FOR ODOUR ENCODING IN THE OLFACTORY LOBE

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SUMMARY

1. Studies were made of quality coding in the olfactory lobe of the insect by recording extracellular action potentials from single cells.

2. Listing cell spectra permits to distinguish two main groups of cells, namely, 'odour specialist' which respond very specifically to biologically important substances and 'odour generalist' which respond to a large variety of odorants (thirty-two compounds) in an excitatory or inhibitory manner, or not responding at all.

3. Among more than fifty cells of the 'odour generalists', very few had similar, or identical, reaction spectra to an arbitrarily chosen set of thirty-two odorants, while the 'odour specialists' are like each other in their response spectra.

4. There was an indication of a regional and layer differentiation of response in the lobe to the sex attractant.

5. 'On', 'on–off', and 'off' response types, as well as several variations on these response types, were found in single units during odour presentations.

6. Differences in patterning of excitation for each of the thirty-two compounds can be readily detected by the comparison of the relative amounts of activity in each of the eighty-one units tested. It is therefore concluded that the mechanism of odour encoding at the olfactory lobe may involve the linear combinations of every olfactory neurone's activity result ing in a unique across-lobe pattern of discharges ('odour code pattern') for each particular odorant.

7. If it follows that odour discrimination by the lobe depends on such differences of 'odour code patterns', it would be possible then to distinguish very many odorants simply by having very many neurones possessing differential odour specificity.

INTRODUCTION

One of the most striking features of an olfactory system is its ability to discriminate a vast number of different odours. Consequently, the search for the manner by which odour quality is encoded by olfactory systems has occupied many researchers.

Adrian (1951) approached the problem by recording the discharges of the mitral cells in the rabbit olfactory bulb. He found that some compounds, such as heptane, were more effective in stimulating the posterior regions of the bulb, while others, such as amyl acetate were more effective anteriorly. Adrian's observations were, in essence, confirmed and extended by Mozell & Pfaffmann (1954), and by Mozell (1958) who found that differential regional responses to various odours were maintained at various levels of concentration. Moulton (1965), by recording simultaneously the multifibre discharges occurring at multiple sites in the rabbit olfactory bulb, found that different odours were able to set up quite distinct patterns of excitation across the olfactory lobe.

In invertebrates, however, very few reports have been published concerning the olfactory responses from single units in the olfactory lobe. Yamada (1968), using microcapillary electrode techniques, first recorded single unit activities from the olfactory lobe of the American cockroach, *Periplaneta americana* (L.). He found three typical types of response of single units to odour stimulation, namely 'on' response, 'on–off' response, and 'off' response. He also indicated the existence of some odour-specific neurones in the lobe. Yamada, Ishii & Kuwahara (1968, 1970), using the crude sex attractant extracted from the virgin females of the American cockroach, found very specific and sensitive neurones to the sex attractant not only in the olfactory lobe of the males but also in those of the females of this species. This was somewhat surprising, because the crude sex attractant elicited a vigorous, sexual behavioural response only in the males.

Accordingly, the work to be reported here is an attempt to elucidate how the activity of each single neurone in the lobe is related to stimulus quality with a view to understanding the mechanism of odour discrimination.

METHODS

Animal preparation

The adults of the American cockroach (*Periplaneta americana*), bred in the laboratory, were used. The insects were anaesthetized with carbon dioxide for several seconds while they were being secured with adhesive tapes and wire hooks on a cork plate so that the head could not be moved. The dorsal aspect of the olfactory lobe was exposed by removing a square section of the dorsal exoskeleton of the frons and tracheations overlying the lobe as shown in Fig. 1.

Chemical preparation

The prepurified sex attractant used in the present experiment was extracted by the same method as that described by Yamada *et al.* (1968). That is, some one hundred virgin females were fed on dog biscuit and water in a glass pot in which filter papers were placed. After 2 weeks the filter papers were soaked in 500 ml. water. The water extract was steam-distilled until 100 ml. distillate was collected. Following salting out, the sex attractant was extracted with 500 ml. ether. The ether solution of the crude sex attractant on filter paper elicited a strong sexual response from the male cockroach when a filter paper was brought near. Prepurified aggregation pheromone

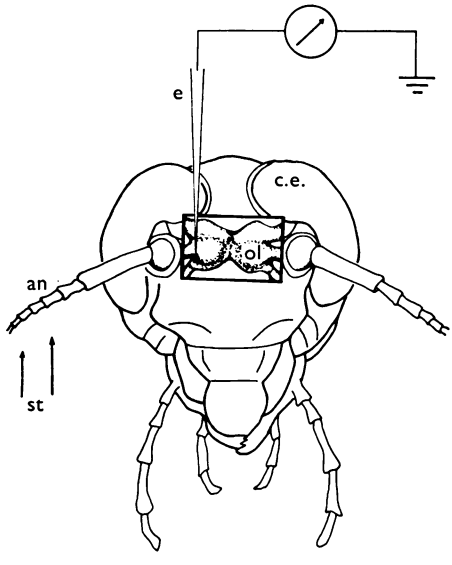

Fig. 1. Anterodosal view of the head of a cockroach prepared for recording from antennal lobe. The brain is shown in a square frame as seen after removal of a flap of frontal cuticle and the inner wall of the frontal air sac. The recording electrode (*e*) is placed in the region (ol) where the microelectrode most frequently registered olfactory activated spikes. c.e., compound eye; an, antenna; st, stimulant air.

of American cockroach, and prepurified aggregation pheromone of German cockroach (*Blattella germanica*) were kindly supplied by Dr Ishii of Kyoto University. These pheromones serve as an attractant to the American cockroach for aggregation. The other chemicals used were cycloheptanone, cyclopentanone, geraniol, ethylene glycol, methyl ethyl ketone, *trans*-2-hexenol, acetic acid, acetone, creosote, clove oil, N-hexyl alcohol, 2-terpineol, cinnamyl alcohol, 2-phenylethanol, *tert.*-amyl alcohol, *trans*-cinnamaldehyde, 1,2-dichloroethane, mentone, dimethyl disulphide, isosafrol, propionic acid, N-butyric acid, formic acid, N-caproic acid, benzyl acetate, iso-amyl acetate-, N-amyl acetate, ethyl carbamate, ammonium water, carbon tetrachloride, benzene, glycerin, paraffin liquid, ethyl ether, chloroform and cedar oil.

Odour stimulation

The olfactory receptors on the antenna were stimulated by a puff from a polyethylene wash-bottle containing a filter paper impregnated with a certain quantity of a odorous substance. The puff technique of stimulation was a very convenient way to test many different odorants on the same preparation.

Electrical recording

Conventional glass capillary micro-electrodes with a tip diameter less than 1 μ, filled with 3 M-KCl solution, were prepared. The electrical resistance of the electrode ranged between 10 and 50 MΩ. The electrode was led, via a cathode follower, to a conventional high gain, dual-beam oscilloscope. Single-unit activity was displayed on one beam. A stimulus marker on the second beam was controlled manually by a microswitch. Thus the markers did not accurately coincide with the colour stimulation. Later experiments avoided this problem by the use of an electric valve and stimulator. These nerve responses were also monitored with an audiomonitor. Under these conditions it was possible to record the nerve activity over a period of hours.

RESULTS

A. General electrical characteristics of olfactory lobe

No electrical changes were seen as the micro-electrode touched the surface of the lobe. Acetic acid stimulation on the antenna evoked a characteristic positive slow potential ('evoked potential') as shown in Fig. 2*A*, but sex attractant failed to elicit any recognizably reproducible responses (Fig. 2*B*). When the micro-electrode was advanced into the lobe, very few spike discharges were spontaneously observed. Generally, the action potentials varied with a prominent positive deflexion, or a prominent negative deflexion, or intermediate shapes, depending on the place of the recording electrode in the lobe. The spikes shown in Fig. 2*C* were initially positive followed by a negative deflexion. As the electrode approached a discharging unit, the positive potential rapidly increased in amplitude whereas the negative part of the spikes remained unaltered. Sometimes the negative part increased more than the positive part of the spike. In this layer the sex attractant produced a negative slow potential accompanied by a burst of impulse discharges during the stimulation (Fig. 2*C*). Acetic acid evoked a positive slow potential with depression of the spike discharge (Fig. 2*D*). In these cases, the results may well be explained in terms of depolarization of the membrane. However, in general, no correlation was observed between the spike initiation and slow potential shapes, perhaps because the slow potentials might come from the summation of a large number of EPSP and/or IPSP around the electrode tip in the lobe. This unit also responded with a slight increase of impulse discharge to generaniol and trans-2-hexenol. Creosote inhibited the spike discharge of this neurone. However, the other forty-one types of odour

stimuli tested failed to facilitate or depress the activity of this neurone. In this paper the word 'facilitation' will be used to indicate an increase in the firing rate of a neurone, while the term 'inhibition' will be used to indicate the contrary. Interestingly, with an electrode in this same region it is nearly always possible to find the units which will respond only to the prepurified sex attractant; in other regions of the lobe, however, it is very

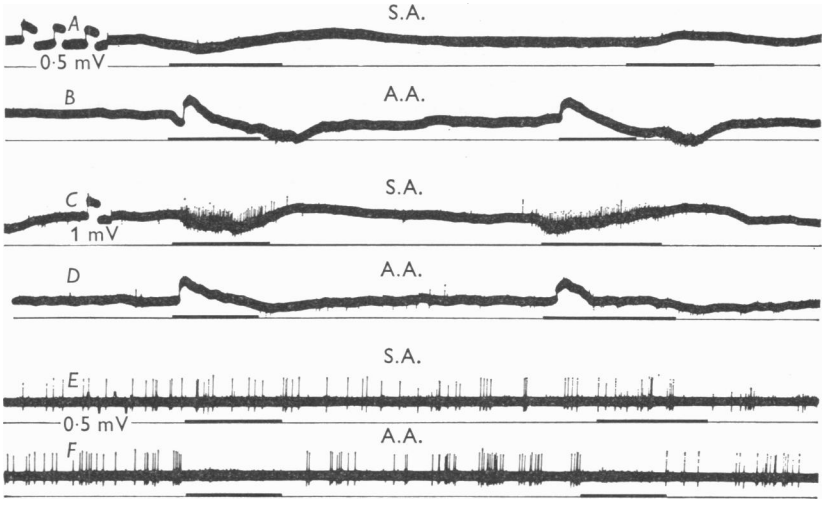

Fig. 2. Extracellular recordings of the electrical activity of the neurones in the olfactory lobe of male adult cockroach (*Periplaneta americana*). The micro-electrode was inserted vertically from the surface (*A*, *B*) through the middle layers (*C*, *D*) toward the bottom of the lobe (*E*, *F*).

Stimulus: prepurified sex attractant of the American cockroach (S.A.) and acetic acid (A.A.) were applied by squeezing the polyethylene bottle. Upward deflexion in this and the following records indicates positivity of the recording electrode.

Stimulus signals are indicated by the horizontal black bar of each lower tracing. Recordings of *A*, *B*, *C*, *D* were made with a longer time constant (0·3 sec).

hard to pick up units which react so specifically. Unitary discharges from deeper layers were usually observed as shown in Fig. 2*E* and *F* in the absence of overt stimulation. These data strongly suggest that a regional (or layer) differentiation of response might occur at the insect olfactory lobe as observed in the rabbit olfactory bulb by Adrian (1951). Fig. 3 shows the characteristic temporal differences in the onset of activity elicited in the two units. The pattern of the spontaneous discharge varied from one unit to another. Some units maintained almost regular rhythmic activity while others discharged at a low irregular rate. In some cases, the stimulus did activate units which were not spontaneously active. The

non-spontaneously or slightly active units were found in the relatively shallow layer of the olfactory lobe, while higher spontaneous rates of discharge were generally recorded in the deeper layer. The frequency of spontaneous activity varied from 0 to 20 impulses/sec.

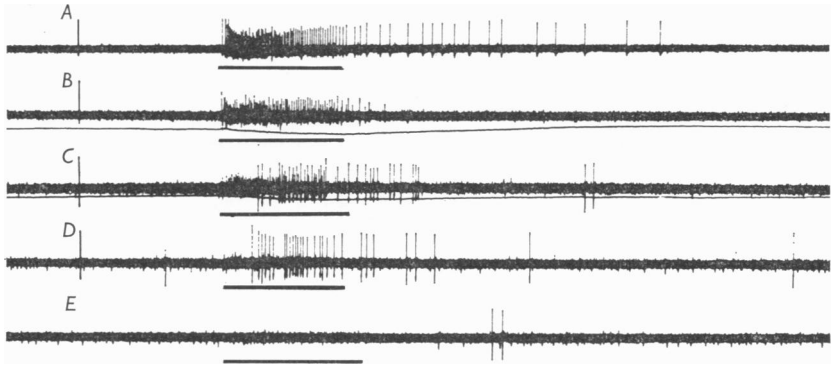

Fig. 3. Illustrations of the electrode penetration process from sex attractant specialist layer (Fig. 3*A*) to other types of cells layer. The spike height of the sex attractant specialist decreases gradually (Fig. 3*B*). At the same time, the other type of spike (large spike) emerges abruptly (Fig. 3*C*), and finally the sex attractant specialist disappears, to be replaced completely by this newly born large spike (Fig. 3*D*). It is interesting that the latency of the large spike to the sex attractant is always much longer than the sex attractant specialist to the sex attractant. Methylethyl ketone failed to produce any effect on these two neurones (Fig. 3*E*). Lower tracings (*D* and *C*) in Fig. 3 show slow potentials.

B. Classification of units according to the response

The activity of single units was recorded in various areas of the olfactory lobe while stimulating the antenna with the thirty-two different odour compounds.

Fig. 4 shows a typical record of the activity of a single unit when the olfactory receptors were stimulated qualitatively with a series of odour compounds. As seen in Fig. 4*A*, there is an increased activity when the stimulation of the prepurified sex attractant is turned on, and this activity was maintained throughout the stimulation. Creosote seems to inhibit the spike discharge of this neurone (Fig. 4*B*). However, the unit failed to respond to other types of odour stimuli (Fig. 4*C*, *D*, *E*, *F*). From this it is clear that this unit is specially sensitive to the prepurified sex attractant. It reacts to a concentration which has no effect on the neighbouring units, and it is not sensitive to the odours which have strong effects on the neighbouring units.

Other typical examples of this kind of specialization were often observed in responses to the aggregation pheromone of the German cockroach (as

seen in Fig. 6, unit nos. 15, 46 and 51 in male column; 15 in female column), menthone (unit no. 2 in male column; nos. 4 and 9 in female column), geraniol (unit no. 3 in male column; 10 in female column), creosote (unit nos. 14 and 31 in male column; 3 and 19 in female column), cycloheptanone (unit nos. 41 and 44 in male column), acetic acid (unit no. 32 in male column), and the aggregation pheromone of the American cockroach (unit no. 56 in male column). In some cases, however, acids and creosote inhibited the activity of neurones specifically (unit nos. 8 and 50 in male column; no. 2 in female column).

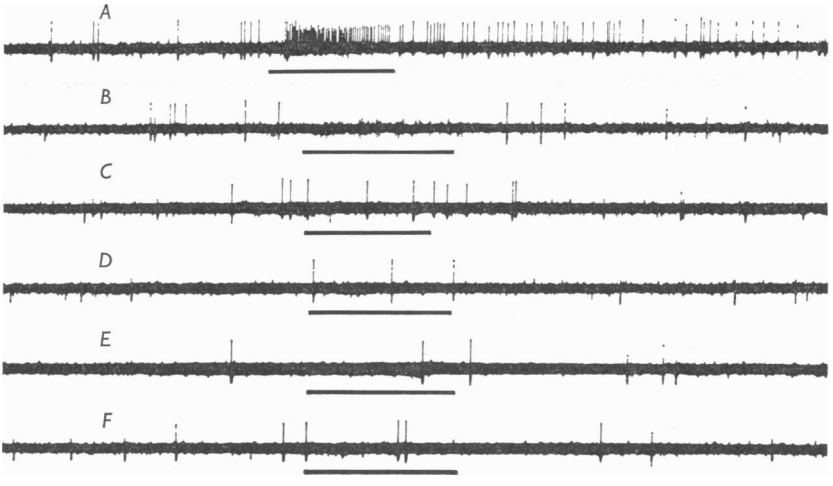

Fig. 4. The response spectra of the sex attractant specialist to an arbitrarily chosen set of compounds.

Among these compounds the sex attractant and aggregation pheromone are known to be biologically meaningful stimulus odours for the insect (Wharton, Miller & Wharton, 1954; Ishii, 1970). Therefore, cells responding specifically to these biologically important substances could be called 'odour specialists' as analogous cells in insect olfactory receptors are termed (Boeckh, Kaissling & Schneider, 1965; Schneider, 1969).

Except for these types of cells ('odour specialists') the bulk of the cells in the olfactory lobe seem to be sensitive, more or less, to an arbitrarily chosen set of odorants since they respond to a given compound in an excitatory or inhibitory manner, or not at all. We call this class of neurones the 'odour generalists' (Boeckh *et al.* 1965). A typical example of 'odour generalists' is seen in Fig. 5 which shows responses evoked in two units (the large spike and the small spike as indicated by arrows). It will be seen that amplitudes of spikes were more or less variable for a given electrode position. These variations could be traced to small intracranial

oscillations of the olfactory lobe which arose from the animal's cardiovascular movements. Four types of odour, iso-amyl acetate, paraffin liquid, creosote and propionic acid, had different effects on two units: iso-amyl acetate excited both neurones (large spike and small spike) to

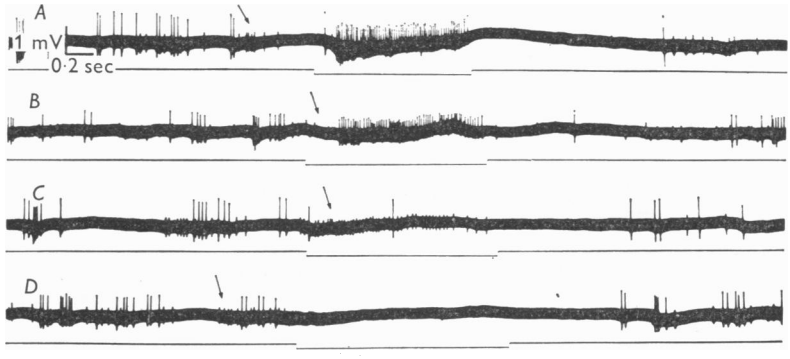

Fig. 5. Differential sensitivities to iso-amyl acetate, paraffin liquid, creosote, and propionic acid are shown in a simultaneous recording of the activity of two 'odour generalists'.

produce a burst of discharges during the period of stimulation. The background impulses were temporarily inhibited immediately after the cessation of the stimulation (Fig. 5*A*). Paraffin liquid also excited the two neurones, but the small spike appeared much faster than large spikes (Fig. 5*B*). Creosote excited the small spike, while inhibiting the large spike (Fig. 5*C*). Propionic acid inhibited the large spike as well as the small one (Fig. 5*D*). Fig. 6 shows the summary of the reactions of the

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Legend to Fig. 6.

Fig. 6. Reaction spectra of single units to an arbitrarily chosen set of odorants. Each vertical line shows the reaction spectra of one unit to a series of compounds. For each odorant, responses at all eighty-one units could be regarded equivalent to those recorded simultaneously from these eighty-one units except for timing in the onset of activity elicited in each unit, because every odour could be thought to be in constant concentrations in every experiment. A.H.A. is aggregation pheromone of the American cockroach. A.H.G. is aggregation pheromone of the German cockroach (*Blattella germanica*) which could attract the American cockroach. In classifying the response, the effect evoked by natural stimulation was denoted as excitatory, inhibitory, or unaffected. Any type of facilitation during stimulation is grouped into the 'excitatory' class, and the word 'facilitation' indicates an increase of impulse frequency irrespective of its being spontaneously active or not, while the term 'inhibition' is used to indicate a decrease of impulse frequency during stimulation or an increase of impulse frequency after stimulation.

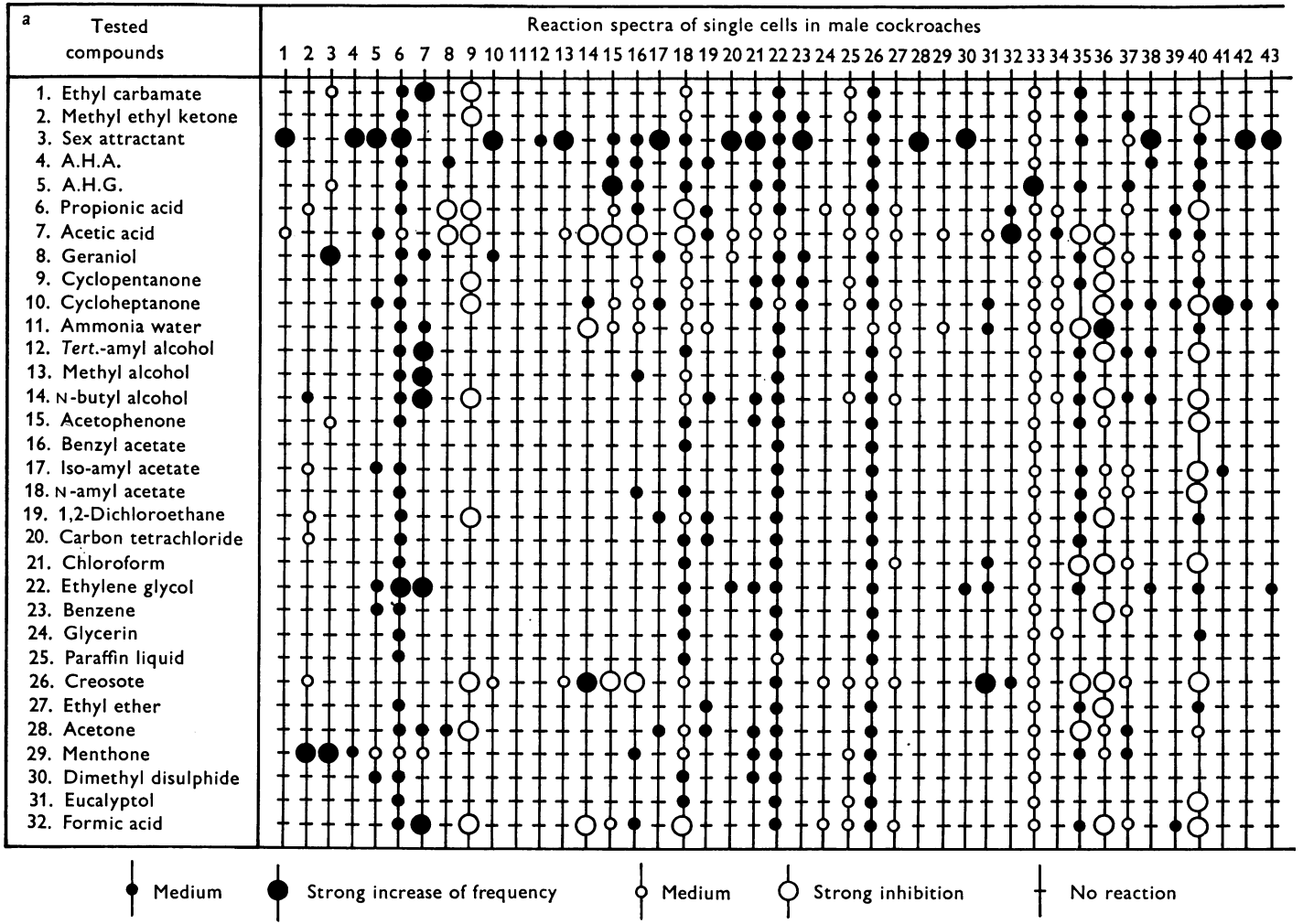

Fig. 6*a*. For legend see opposite page.

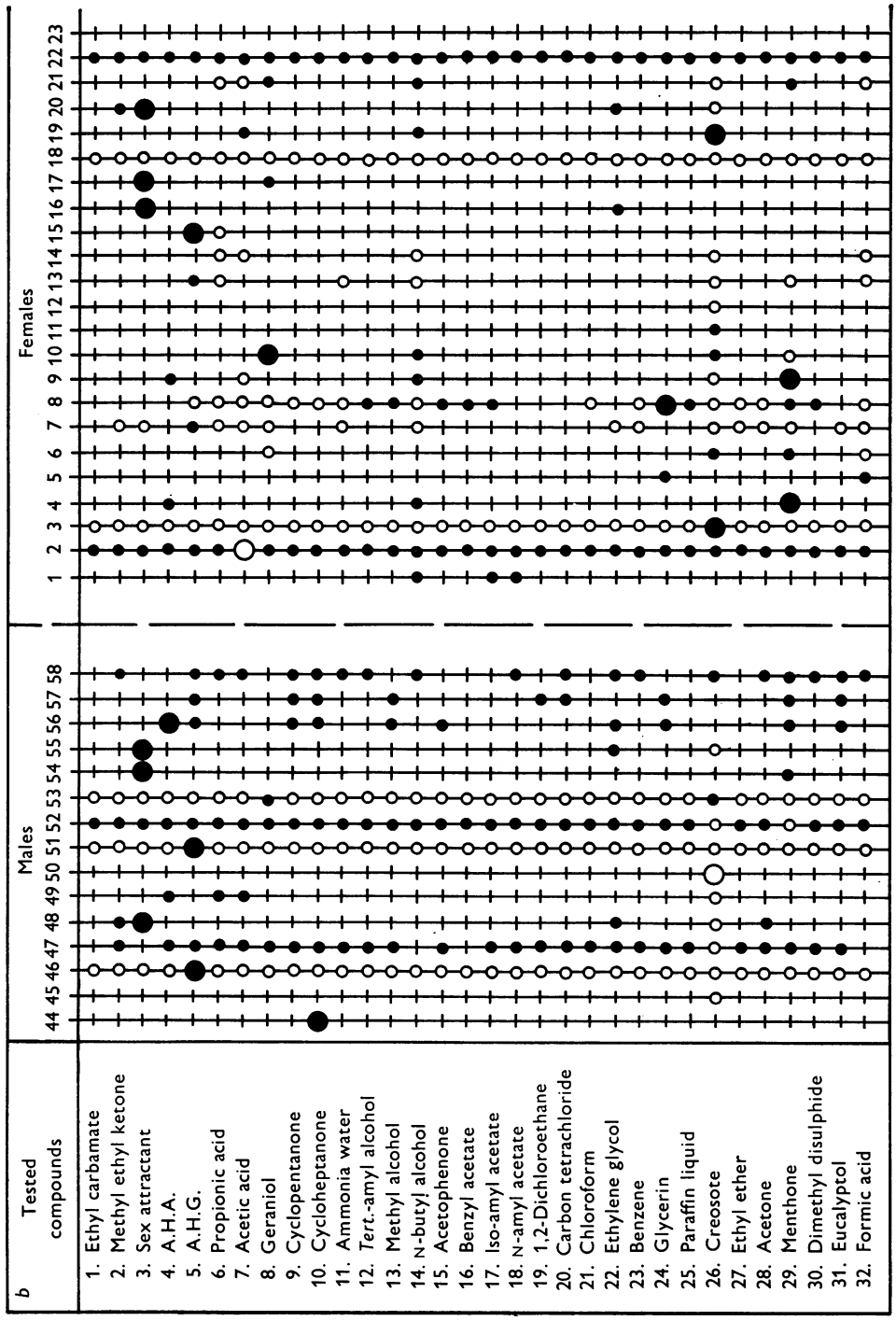

Fig. 6*b*. For legend see page 134.

single units tested in both sexes to an arbitrarily chosen set of odorants. The spectra of the 'odour generalists' overlap considerably, but there is still a striking spectral variability from cell to cell. On the other hand, the reaction spectra among 'odour specialists', for example, the sex attractant specialists (unit nos. 1, 4, 5, 10, 13, 17, 20, 21, 23, 28, 30, 42, 43, 48, 54 and 55 in male column; 16, 17, and 20 in female column) and aggregation pheromone specialists (unit nos. 15, 33, 46, and 51 in male column; 15 in female column) are very similar to each other. It should be noted here that in both sexes there were often found some units which did not respond at all to the odour stimulations (e.g. unit no. 23 in female column), or which responded equally to the every odour stimulation, even to the control air puffs. Although the number of odours tested was limited, it is highly probable that the former are neurosecretory cells, and the latter are mechanical sensory cells. This latter response is classified into two types, the 'inhibitory' type (e.g. unit no. 18 in female column) and the 'excitatory' type (e.g. unit no. 22 in female column).

C. Response patterns of single cells to odours

There are roughly two classes of spike discharges, namely spontaneously active or inactive neurones, although it is very hard to discriminate when a unit fires in very low frequency.

In the first class, various patterns of responses were observed:

(*a*) facilitation which soon ceased despite continued stimulation (Fig. 7*A*),

(*b*) facilitation which ended a few seconds after the end of the stimulus (Fig. 7*B*),

(*c*) facilitation during the stimulus followed by a period of inhibition following end of the stimulus (Fig. 7*C*),

(*d*) facilitation during the stimulation followed by a period of inhibition which itself was followed by a rebound or facilitation (Fig. 7*D*),

(*e*) inhibition which could outlast, for a few seconds, the duration of the stimulus (Fig. 7*E* large spike),

(*f*) inhibition during the stimulation and facilitation (or rebound) afterwards (Fig. 7*F*),

(*g*) no facilitation, or no inhibition during or following the odour stimulation (Fig. 7*G*).

In the second class, too, varieties of response patterns were observed:

(*a*) facilitation terminating quickly despite continued stimulation (Fig. 8*A*),

(*b*) facilitation continuing during stimulation (Fig. 8*B*),

(*c*) facilitation which could outlast, for a few seconds the duration of the stimulus (Fig. 8*C* and the small spike of Fig. 8*D*),

(*d*) facilitation throughout the stimulation accompanied by a period of silence (inhibition) followed by refacilitation (rebound) (Fig. 8*D*),

(*e*) facilitation at the very beginning and at the end of the stimulus (Fig. 8*E*),

(*f*) facilitation after the stimulation (inhibition followed by rebound) (Fig. 8*F*),

(*g*) no facilitation during and/or end of the odour stimulation (Fig. 8*G*).

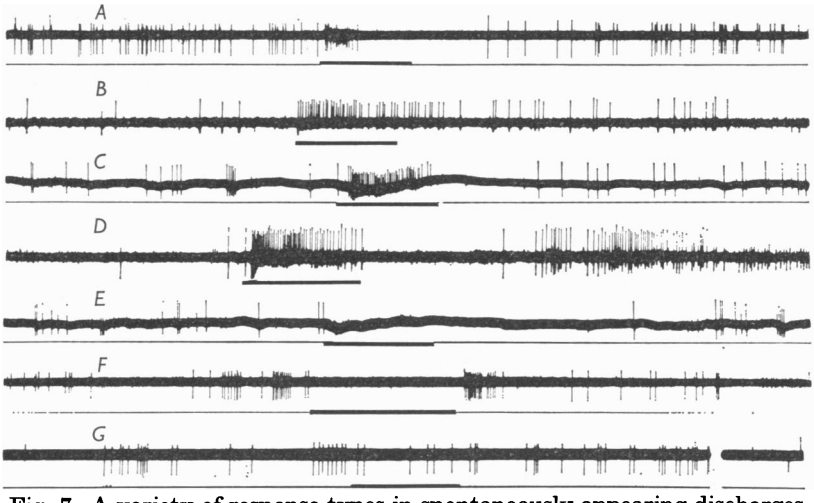

Fig. 7. A variety of response types in spontaneously appearing discharges. *A*, *B*, and *C*: 'on type'. *D*: 'on–off type'. *E* and *F*: 'off type'.

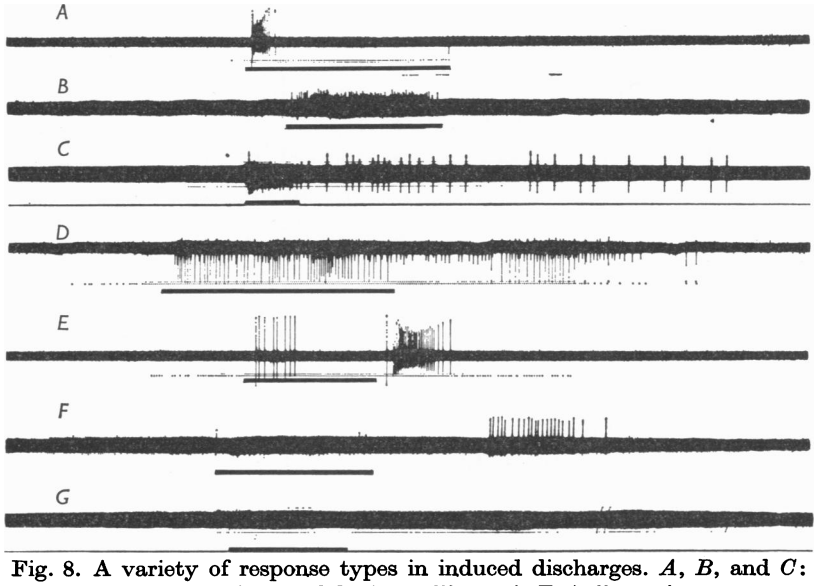

Fig. 8. A variety of response types in induced discharges. *A*, *B*, and *C*: 'on type'. *D* and *E*: 'on–off' type'. *F*: 'off type'.

Thus, it was shown that there are at least six or seven patterns of responses in each class. Fundamentally, all the responses described above might be roughly grouped into four categories as reported by Yamada (1968): namely, an 'on' response which shows some facilitation during stimulation; an 'on–off' response which shows some facilitation during stimulation and after stimulation; an 'off' response which shows some inhibition during stimulation and facilitation afterward; and no change of nerve activity by stimulation. Most of these patterns of discharges were already found in the olfactory bulb as well as in the olfactory epithelium of vertebrates (Shibuya, Ai & Takagi, 1962; Mancia, Baumgarten & Green, 1962; Takagi & Omura, 1963). However, it is not yet clear whether information of this kind is pertiment in odour discrimination, or whether it represents an incidental by-product of bulbar activity.

DISCUSSION

Of the highest interest to the present discussion is the demonstration of neurones which are highly specific to biologically important substances ('odour specialists') at the level of secondary neurones (olfactory lobe).

This high specificity is firmly supported by quantitative experiments using high concentrations for possible effective chemicals (Yamada, Ishii & Kuwahara, 1970). A human observer watching only the spike traces coming on the screen of the cathode-ray oscilloscope can easily identify the odorant eliciting any given response in each specialist once he knows the code. Thus it seems that the animal must use these 'specialists' to transmit only the information of the biologically active substances to the higher centre of brain.

An immediate problem of this 'odour specialists' system arises from the fact that females of the cockroach fail to respond with the full sexual excitement display (such as fluttering or outspread wings and extended abdomen) of the sex attractant, although they, too, have a 'sex attractant specialist' system similar to that found in males.

There are several alternatives by which this somewhat confusing picture can be explained. (1) The idea of the 'odour specialists' system does not exclude the very probable case in which other olfactory cells in addition to the 'sex attractant specialists' respond to the sex attractant in an excitatory or inhibitory manner. For instance, some 'odour generalists' and the 'A.H.G. specialists' in Fig. 6 responded to the prepurified sex attractant. Therefore, if it is assumed that the sort of combination which every olfactory neurone makes has vital importance in determining odour quality, this problem can be easily explained by simply assuming the differences in the 'odour generalists' and the other 'odour specialists'

between both sexes, even when the 'sex attractant specialists' in the lobe are the same between both sexes. (2) It would be reasonable to assume that the ways of connectivity of neuronal network at the highest centre (probably corpora pedunculata) might be generally different in both sexes. Therefore, even when the inputs from the lobes are the same between both sexes, the outputs from the brains of both sexes will be different. In other words, at the level of secondary neurones the female could receive the sex attractant in the same way as the male does, but at the highest centre the female perceives the sex attractant in a different way from the male. (3) The answer to this problem might come partly from the differences of centrifugal pathways which are connected with effector organs in either sex. If the male has different centrifugal nervous pathways from that of the female, it would be logical if both sexes had different patterns of behavioural responses to the sex attractant, even though the outputs from the brain were similar. (4) The so-called 'sex attractant specialist' in the female might be other 'odour specialists' which respond specifically to a certain kind of odour compound present in the prepurified sex attractant, because the prepurified sex attractant usually contains many odorous compounds.

The biological meaning of the 'sex attractant specialists' system of females in this species cannot be discussed in detail here until the behavioural responses of the females to the pure sex attractant has been clearly identified.

For the aggregation pheromone specialists, it was assumed at first that there might be 'specialists' for the aggregation pheromone of the American cockroach, and it was therefore rather surprising that there are 'specialists' for the aggregation pheromone of the German cockroach, in the lobe of the American cockroach, and very few 'specialists' for the American cockroach's aggregation pheromones. However, it should be noted that, behaviourally, the aggregation pheromone of the German cockroach will attract the American cockroaches (Ishii, 1970).

On the other hand, the 'odour generalists' have a specific spectrum of responses which is the sum of the responses to all compounds. Therefore, their response spectra vary greatly from cell to cell (Fig. 6). Very few had similar, or identical reaction spectra to an arbitrarily chosen set of odorants. On the contrary the 'odour specialists', such as the 'sex attractant specialists', have very similar response spectra (see Fig. 6). In the light of these observations it is apparent that the absolute amount of activity in any one 'odour generalist' cannot by itself encode quality.

How may the olfactory lobe, with the properties outlined above, encode odour quality? In Fig. 5 the four stimuli tested can be discriminated if both the small and large amplitude spikes are considered simultaneously: when the discharge of the small spike is high, in conjunction with the

response of the large spike, the stimulus signalled is creosote (Fig. 5*C*); when both the small and large spike discharge disappear, the stimulus signalled is propionic acid (Fig. 5*D*); when both the discharges of the small and the large spikes are high, the stimulus signalled is iso-amyl acetate or paraffin liquid; these are discriminated by a temporal difference as shown in Fig. 5*B* (Paraffin liquid). The onset of activity of the large spike is much more delayed than the small spike, which is, however, almost covered by background noise and hard to see in Fig. 5*B*, whereas in Fig. 5*A* (iso-amyl acetate) the large spike appeared almost simultaneously with the small spike.

Thus, the stimulus odours in Fig. 5 can be analysed without confusing them with stimulus intensity. In this way, if the types of differences revealed here are extended over all olfactory neurones including the 'odour specialists' in the lobe, a great number of odorants could be easily distinguished simply by having very many fibres. This same coding mechanism was proposed by Pfaffmann (1955, 1959), Erickson (1968), and its mathematical model by Yamada, Yomosa & Hasegawa (1970) to account for quality coding in primary taste and olfactory receptors. Implicit in this idea of coding is the assumption that the relative amounts of activity elicited by different chemicals in each cell will not be altered significantly by changes in stimulus concentration. This assumption had been examined and confirmed in the olfactory receptors by O'Connell & Mozell (1969). In this scheme the transformation from chemical compound space to smell space involves the linear combinations of the space-time aspect of every olfactory neurone's activity resulting in a unique across-lobe pattern of discharge ('odour code pattern') for each particular odorant. The characteristic temporal differences in the onset of activity in Figs. 3 and 5 as well as during odour presentation as shown in Figs. 8 and 9 will certainly give the neural basis for the olfactory lobe to elicit many discriminable 'odour code patterns' across the lobe for each compound. However, even by ignoring these obvious differences in temporal aspect of response, differences in patterning of excitation for each of the thirty-two compounds including the sex attractant and aggregation pheromone could be readily detected by the comparison of the relative amounts of activity in each of the eighty-one units tested in Fig. 6. For example, ethyl carbamate and methyl ethyl ketone evoked quite similar responses at seventy units; however, eleven units such as unit nos. 3, 7, 40, etc., responded in different ways to these two compounds. It is of interest to note in this analysis that some compounds such as iso-amyl acetate and N-amyl acetate produced very similar patterns of excitation, whereas others, e.g. acetic acid and sex attractant, produced quite different patterns.

From these observations it is reasonable to assume that the sex attractant and the aggregation pheromone could also be encoded by the 'odour

code patterns' developed across the total ensemble of olfactory neurones in the lobe.

However, this does not decrease the significance or role of the 'odour specialists' in the odour discrimination. For example, only the 'sex attractant specialists' can produce the 'odour code pattern' for the sex attractant very clearly and strongly, for the sex attractant excites the 'sex attractant specialists' very strongly, but has very little or no effect on other neurones as seen from Fig. 6. Accordingly, the 'odour code pattern' of the sex attractant would be very different from other 'odour code patterns', so that the insect clearly recognizes and discriminates the sex attractant in the living environment where many kinds of odorous molecules are contained. For this reason, it might be said that it is as if the sex attractant is coded in terms of activity or inactivity in the 'sex attractant specialists'. Here it is perhaps worth describing the olfaction of vertebrates. So far, in the olfactory system of vertebrates, many substances have been used in searching for an odour-specific neurone. Adrian (1951) found a region in the cat olfactory bulb which was particularly sensitive to trimethylamine (present in decaying fish). Hara, Ueda & Gorbman (1965) reported that in homing salmon, water from the home pond produced a vigorous response of high amplitude in the olfactory bulbs, whereas various natural waters from nearby sources other than the home pond produced little or no change in spontaneous electroencephalographic patterns. These findings suggest the possibility that the vertebrates could have the specialized neurones for biologically important substances like the 'odour specialists' of insects. Consequently, it does not seem too unrealistic to think that the olfactory nervous system, in all its evolutionary wisdom, could come to take advantage of this 'odour hot-line cable (specialist) system' for such biologically important conversation with nature. Gesteland, Lettvin & Pitts (1965) could not find such an odour-specific neurone like insects' 'odour specialists' in the frog's nose. Instead, almost every odour seems to affect almost every receptor in one way or another. In this respect, the frog's olfactory receptors are like the 'odour generalists' in insects (Schneider, Lacher & Kaissling, 1964).

Thus, it is strongly suggested that the 'odour code patterns' could exist and play a vital role in determining odour quality at the level either of receptors or higher order in the olfactory system of both invertebrates and vertebrates.

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